

possibility of its containing such substances as may increase sperm motility or affect some unknown activity either of the sperms or of the female reproductive organs.

At any rate, the waxy plug may be regarded as a fertility factor. As a decrease in ejaculatory bulb secretion has been observed following repeated matings (1), variations in fertility rates may be caused not only by a reduction in accessory gland secretion (2) but also by inadequate activity of the ejaculatory bulb.

Finally, it must be definitely said that, on the strength of all the findings reported, the plug which is found in the uterus after a mating has simply nothing to do with the fluid secretion which Patterson (3) and other workers have reportedly noticed inside the genital duct of the *Drosophila* genus as a reaction to insemination. The fact must not be overlooked, indeed, that the plug is present after 5 to 8 minutes since mating beings, before any sperm is present and before any reaction is exhibited by the female genital duct's mucosa - and, more important still, the fact should be remembered that the plug is formed by the ejaculatory bulb secretion. This does not mean that a reaction to insemination may not occur, as noted particularly with interspecies matings, but merely that the waxy plug should not be regarded as the product of such a reaction. At this stage, two different assumptions should be investigated: either the waxy plug is the only material contained within the female genital duct of *D. melanogaster* besides the sperm after mating, or, together with it, the duct also contains the fluid secretion produced by reaction to insemination. Should the first hypothesis be verified, the plug and fluid secretion would be one and the same thing, and the actual existence of a secretory activity primed by insemination would then call for further investigation. As reaction to insemination is generally regarded as an effective selection mechanism in interspecies matings in the *Drosophila* genus, the finding we have just reported would seem to acquire a general biological and genetical significance as well as to warrant further, more systematic, investigations.

References: 1) Bairati, A., 1968, Structure and ultrastructure of the male reproductive system in *D. melanogaster* Meig. 2° - The genital duct and accessory glands. *Mon. Zbol. Ital. (n.s.)* 2: 105-182. 2) Perrin-Waldemer, C., 1965, Biologie de la reproduction du male et des spermatozoides chez *D. melanogaster*. *Ann. Biol. Anim. Bioch. Biophys.* 6: 553-585. 3) Patterson, J. and Stone, W., 1952, *Evolution in the Genus Drosophila* MacMillan Co., New York.

Gateff, E. and H. A. Schneiderman. Case Western Reserve University, Cleveland, Ohio. Long term preservation of imaginal disc cell lines at low temperature.

When lines of imaginal disc cells with novel developmental capacities arise in the course of in vivo culture (Hadorn, 1965) one wants to maintain them for further study. To do this involves repeated subculturing in adults at intervals of one or two weeks. The time intervals can be lengthened to a month by implanting the tissue fragments into adults of *D. virilis* which are larger. But as more and more novel lines arise the investigator is forced to destroy certain lines because of the difficulties of keeping them continuously subcultured. We have modified a preservation technique originally designed to preserve bacterial cultures at low temperatures (Bouroncle, 1965).

The preserving medium is a solution of 75% *Drosophila* Ringer's, 15% calf serum and 10% dimethylsulfoxide. One ml. of this solution is placed in a sterile ampoule. The adult abdomen containing the fragment of tissue to be preserved is separated from the thorax and cut open at the posterior tip. This leaves the abdomen open at both ends. The abdomen containing the imaginal disc fragment is placed in the vial which is then sealed in a flame and placed in a dry-ice-acetone bath at  $-80^{\circ}\text{C}$  and then into a  $-78^{\circ}\text{C}$  deepfreeze.

When the tissues are needed, the ampoule is thawed in a  $40^{\circ}\text{C}$  waterbath and then cut open. The abdomen is washed three times in Ringer's and the implanted tissues may now be used. These frozen tissues retain the capacity to grow when cultured in adult abdomens and to differentiate when implanted into larvae. The longest time tissues were kept at low temperatures was three and one-half months. When thawed, both the frozen implanted tissues and the organs of the frozen adult host abdomens appeared normal.

Hadorn, E. 1965. *Brookhaven Symp. Biol.* 18: 148-161. Bouroncle, B. A. 1965. *Proc. Soc. Exp. Biol. and Med.* 119: 958-961.